Insecticide, sugar, and diet effects on feeding and mortality in *Rhagoletis indifferens* (Dipt., Tephritidae)

W. L. Yee

United States Department of Agriculture, Agricultural Research Service, Yakima Agricultural Research Laboratory, Wapato, WA, USA

Keywords
western cherry fruit fly, neonicotinoids, feeding duration, paralysis and mortality

Correspondence
Wee L. Yee (corresponding author), USDA-ARS, Yakima Agricultural Research Laboratory. Konnowac Pass Road Wapato, WA 98951, USA. E-mail: wee.yee@ars.usda.gov

Received: September 12, 2008; accepted: October 26, 2008.

doi: 10.1111/j.1439-0418.2008.01359.x

Abstract

The effects of spinosad bait and various insecticides, the presence of sugar in insecticides, and diet on feeding responses and mortality in western cherry fruit fly, *Rhagoletis indifferens* Curran (Dipt., Tephritidae), were determined. Numbers of feeding events on insecticides with sugar were greater than on insecticides alone, but there was only a small effect of diet on feeding responses to insecticides with sugar. Feeding durations on imidacloprid, thiamethoxam and acetamiprid with sugar were shorter than on sugar water and spinosad bait, as the neonicotinoids paralysed flies quickly. Flies that fed on sugar only (nitrogen-starved) suffered higher mortalities when exposed to spinosad, thiamethoxam and azinphos-methyl than to imidacloprid, acetamiprid and indoxacarb, and mortality in between these two groups of treatments when exposed to spinosad bait. Mortalities were greater when sugar was added to insecticides, and were higher in nitrogen-starved than fully-fed (yeast extract + sugar fed) flies. Flies that fed once on thiamethoxam were killed more quickly than those that fed once on spinosad bait and spinosad. Results suggest that thiamethoxam is comparable to spinosad in its effects on mortality, and that using it with sugar in bait may also have similar results as using spinosad bait or spinosad. One benefit of using thiamethoxam with sugar may be that it kills flies more quickly, before they can oviposit, than spinosad bait, although whether a fly will feed on it may depend on how much sugar or nitrogenous food it has eaten.

Introduction

Western cherry fruit fly, *Rhagoletis indifferens* Curran (Dipt., Tephritidae), is the major quarantine insect pest of sweet cherry, *Prunus avium* (L.) L., in the Pacific North-west of the United States. Because of the zero tolerance for larvae in commercial cherries (State of Washington Department of Agriculture, Permanent Order No. 1099, effective 30 September 1968), cherry growers in this region continue to use insecticide products as their primary method for fly control, but effective organophosphate insecticides used in the past are no longer options (Food Quality Protection Act 1996; Reissig 2003). In recent years, spinosad bait (GF-120® Naturalyte® Fruit Fly Bait, Dow AgroSciences, Indianapolis, IN), composed of 0.02% (w/v) spinosad, protein, sugar and other ingredients (Thomas and Mangan 2005), has gained popularity for use in control of *R. indifferens* (Warner 2008). The effectiveness of spinosad bait is dependent in part on fly feeding responses, which is related to hunger, as spinosad is more toxic to flies when ingested than when topically applied (Yee and Chapman 2005). Despite its effectiveness, spinosad bait does not always eliminate infestations of *R. indifferens* (Yee and Alston 2006; Yee 2007).

Spinosad bait and spinosad (without bait) are two of six materials listed for use against *R. indifferens*

(Washington State University 2008). These include the neonicotinoid imidacloprid, which like spinosad has a favourable mammalian toxicity profile (Tomizawa and Casida 2005). Use of neonicotinoids in addition to spinosad bait and spinosad is desirable for several reasons. Although spinosad is recommended for control of pest lepidopteran leafrollers on cherry (Ministry of Agriculture and Lands 2006), some populations of leafrollers have developed resistance against spinosad (Washington State University 2008). Also, spinosad bait has been associated with phytotoxicity on leaves of some sweet cherry varieties (DeLury et al. 2008). Imidacloprid and other neonicotinoids are toxic to flies (Wright et al. 1999; Ayyappath et al. 2000; Reissig 2003; Barry and Polavarapu 2005; Yee and Alston 2006), even though for the blueberry maggot, *Rhagoletis mendax* Curran, some neonicotinoids seemed less toxic than spinosad (Barry and Polavarapu 2005).

Use of sugar-neonicotinoid baits, as of spinosad bait, to entice flies to feed would reduce the amount of insecticide dispensed into the environment compared with use of insecticides as cover sprays. Sugar (sucrose) is a key factor stimulating feeding in tephritid fruit flies (Duan and Prokopy 1993) and when mixed with insecticides can increase mortality compared with insecticides alone (Reissig 2003). Sugar is also a major component of spinosad bait (Thomas and Mangan 2005; Yee and Chapman 2005). The proteinaceous component of the bait stimulates feeding in tropical fruit flies (Moreno and Mangan 2003), but it is unclear if it stimulates more feeding than sugar alone in *R. indifferens*.

In addition to sugar, another factor that may affect whether flies feed on insecticides is diet, defined here as feeding on sugar only or nitrogenous + sugar food before insecticide exposure. In *R. indifferens*, more flies that had fed on sugar only than on sugar + yeast extract responded to spinosad bait (Yee 2006). Diet could affect responses to sugar-neonicotinoid baits by altering activity levels.

If sugar in insecticides and diet affect feeding, they should also affect mortality. The effects of sugar, diet and neonicotinoid insecticides on mortality of *R. indifferens* have not been studied, although effects of neonicotinoid insecticides on mortality are known for other species (Wright et al. 1999; Ayyappath et al. 2000; Reissig 2003). Relative patterns of feeding and mortality effects among insecticides may differ depending on the knockdown ability and toxicities of the insecticides (Barry and Polavarapu 2005).

In this study, feeding responses of *R. indifferens* to spinosad bait and neonicotinoid insecticides were determined. Several hypotheses were tested: (1) feeding responses are greater to insecticides with added sugar; (2) diet affects feeding responses; (3) mortalities of flies are greater in flies exposed to insecticides containing sugar and (4) diet affects mortality.

Materials and Methods

Larval-infested cherries were collected in Kennewick, Richland and Yakima, WA in June and July 2007. Larvae that emerged from fruit dropped into tubs, where they pupated. Pupae were stored in cups with moist soil and held at 3–10°C for 6–10 months before being transferred to 27°C, 30–40% relative humidity (RH), and under 16 h of light of ~1200–1300 lumen/m^2 for adult emergence. Flies were held under these conditions before experiments and were kept in 1.9-liter paper containers (10.5 cm high × 16.2 cm diameter) with a diet of either 5% sugar (w/w) on cotton wicks (nitrogen-starved from eclosion) or yeast extract + sugar (fully-fed from eclosion) [20% yeast extract (EMD Chemicals, Inc., Gibbstown, N.J.) + 80% dry sugar (w/w)] on paper strips. Water was provided on cotton wicks. Flies were aged to 6–8 days for testing. The term 'nitrogen-starved' was used instead of 'protein-starved' because recent work has established that yeast extract has very little protein in it although it appears to be 'nitrogen-rich' (Barry et al. 2007).

Feeding responses to insecticides

In experiment 1, insecticides were either mixed in 20% sugar (sucrose) solution (v/v) or in water alone. No sugar was added to the pre-existing sugar in spinosad bait, which when undiluted has 30% sugar (fructose, glucose and sucrose, w/w) (Yee and Chapman 2005). Treatments were (1) water; (2) 40% spinosad bait (v/v), with ~13% sugar (96 ppm ai, in mg/l); (3) spinosad (Entrust®, Dow AgroSciences, Indianapolis, IN) (36 ppm); (4) imidacloprid (Provado®, Bayer CropScience, Research Triangle Park, NC) (30 ppm); (5) thiamethoxam (Actara®, Syngenta Crop Protection, Greensboro, NC) (103 ppm); (6) acetamiprid (Assail® 70 WP, Cerexagri-Nisso LLC, King of Prussia, PA) (178 ppm); (7) indoxacarb (Avaunt®, E. I. du Pont de Nemours and Company, Wilmington, DE) (135 ppm ai) and (8) azinphos-methyl (Guthion® 50W, Gowan Company, Yuma, AZ) (899 ppm ai). Spinosad is a naturalyte insecticide; imidacloprid, thiamethoxam and acetamiprid are neonicotiniods; indoxacarb is an oxadiazine; and azinphos-methyl is an organophosphate. Rates used were those on product labels.

Three tests were conducted to examine feeding responses of nitrogen-starved and fully-fed flies. In tests 1 and 2, nitrogen-starved flies were offered insecticides with and with no sugar, respectively. In test 3, fully-fed flies were offered insecticides with sugar. Flies were not starving at the time of tests, as 83.3% of nitrogen-starved and fully-fed flies (n = 30) survived to 20–22 days.

For each test, a female or male fly was introduced into a 5.0 cm × 1.4 cm glass vial. A 2.5 μl drop of water or insecticide solution was then applied onto the centre of it using a micropipette. The vial was then plugged with cork, and placed on light blue latex to make the water or insecticide drop more visible. Percent of flies that fed was recorded. Numbers of feeding events and feeding durations over 5 min for a fly were recorded by continuously observing the fly and using a timer. Feeding was mouth contact with a drop. Observations in tests 1 and 2 were conducted at 21°C and test 3 at 25–27°C at ~1100 lumen/m^2 over 8-h periods (0900–1700 h) beginning at 5 h after lights-on during the 16 h light cycle. For the controls and each treatment, at least 20 males and 20 females were tested.

Mortality of flies exposed to insecticides

In experiment 2, treatments and rates used were the same as in experiment 1. Fifteen male and 15 female flies inside a 1.9-liter paper container were exposed to a 9.0 cm diameter × 1.4 cm high clear plastic dish with 10 fresh drops of 10 μl water or insecticide with or without 20% sugar for 2 h. At 2 h, each dish was removed and one sugar cube was placed in the container. Numbers of paralysed or dead male and female flies were recorded immediately after insecticides were removed (0 h) and at 24 and 48 h. Mortality has also been assessed at 48 h in other work (Reissig 2003). Paralysed flies could move their legs but were unable to walk or were motionless. Dead flies did not move when probed. It was sometimes difficult to determine if a fly was dead, so throughout the results, 'paralysis/mortality' is used. There were five replicates of each treatment.

Paralysis and recovery of flies after feeding on insecticides

In experiment 3, effects of feeding on sugar water, spinosad bait, and spinosad, imidacloprid, thiamethoxam and acetamiprid with sugar (same rates as in experiments 1 and 2) on paralysis and recovery of flies were determined, to help explain results of experiment 2. Flies were held on 5% sugar and tested at 6–10-days old inside 5.0 × 1.4 cm glass vials at 21°C. A 10 μl drop of solution was placed ~1–5 mm in front of a fly so it could quickly find the drop, unlike in experiment 1. Duration of one feed was recorded. The fly was then transferred to a clean vial and held at 27°C. Paralysis or death was recorded ≤1 min (0 h) and at 2 and 24 h after feeding. There were 17–22 males and females in the control and each treatment.

Statistics

In experiment 1, percentages of male and female flies that fed were analysed with Fisher's exact test. Differences in percentage responses among treatments and among the three tests were analysed using chi-squared test. For all three tests, numbers of feeding events were square-root $(y + 0.5)$-transformed and feeding durations were log (y)-transformed before being subjected to three-way analysis of variance (ANOVA), with insecticide, sugar in insecticide and diet as factors. Separate one-way ANOVAS also were performed for each insecticide across tests and for all insecticides within a test. Fisher's least significant difference (LSD) test was used for pairwise comparisons (SAS Institute 2004).

In experiment 2, paralysis/mortalities were arscine($\sqrt{y}$)-transformed and subjected to four-way repeated measures ANOVA (insecticide, sugar in insecticides, diet and time), with repeated measures on time (spinosad bait was excluded here because there was no spinosad bait without sugar). Significant interactions among factors do not invalidate further analyses of factors, but indicate that each should be looked at separately as a simple effect (Schabenberger 1998). This was accomplished using the SLICE command in the Statistical Analysis System (SAS), followed by LSD tests (SAS Institute 2004). Simple effects within insecticide, sugar diet and time factors were determined. Paralysis/mortalities among insecticides within sugar (including spinosad bait) and no sugar treatment groups were compared using one-way ANOVA followed by LSD tests.

In experiment 3, feeding durations [log (y) transformed] were analysed using two-way ANOVA (sex and insecticide), and percentage paralysed or dead males and females within treatments analysed using Fisher's exact test. A Tukey-type multiple comparison test among proportions (Zar 1999) was used to test for treatment differences within times.

Results

Feeding responses to insecticides

There were no differences between sexes in percentage responses within each insecticide treatment in tests 1, 2 and 3 (Fisher's exact test, P = 0.2293–1.000) (except for azinphos-methyl in test 3), so data from sexes were combined. There were also no differences in percentage responses among treatments in any of the three tests. In test 1 (χ^2 = 6.5; d.f. = 7; P = 0.4824), the overall response was 40.4% (n = 324). In test 2 (χ^2 = 11.1; d.f. = 7; P = 0.1358), the overall response was 12.8% (n = 321). In test 3 (χ^2 = 6.5; d.f. = 7; P = 0.4824), the overall response was 47.4% (n = 371). The percentage responses in tests 1 and 3 were greater than in test 2 (χ^2 = 63.1; d.f. = 1; P < 0.0001 and χ^2 = 96.1; d.f. = 1; P < 0.0001, respectively), whereas those in tests 1 and 3 did not differ (χ^2 = 3.4; d.f. = 1; P = 0.0635), indicating the presence of sugar in insecticides increased feeding.

For numbers of feeding events (table 1), there was no insecticide effect (F = 1.9; d.f. = 7, 954; P = 0.0695), a sugar in insecticides effect (F = 79.5; d.f. = 1, 954; P < 0.0001), with no insecticide × sugar interaction (F = 1.3; d.f. = 6, 954; P = 0.2600) and no diet effect (F = 0.01; d.f. = 1, 954; P = 0.9144) and no insecticide × diet interaction (F = 0.4; d.f. = 7, 954; P = 0.8698). There were more feeding events by nitrogen-starved flies on insecticides with than without sugar for every insecticide treatment (table 1). Fully-fed flies fed more often on insecticides with sugar than nitrogen-starved flies on insecticides without sugar (table 1).

Table 1 Mean numbers of feeding events ± SE of *Rhagoletis indifferens* exposed to insecticides with and without 20% sugar over 5-min periods

Treatment	Nitrogen-starved flies: Treatment with sugar	Nitrogen-starved flies: Treatment without sugar	Fully-fed flies: Treatment with sugar
Water	0.90 ± 0.19aA	0.08 ± 0.04bA	0.92 ± 0.12aA
Spinosad bait*	0.48 ± 0.12aA	Not tested	0.50 ± 0.12aA
Spinosad	0.53 ± 0.12aA	0.07 ± 0.04bA	0.52 ± 0.09aA
Imidacloprid	0.53 ± 0.09aA	0.08 ± 0.04bA	0.38 ± 0.07aA
Thiamethoxam	0.49 ± 0.09aA	0.15 ± 0.06bA	0.49 ± 0.07aA
Acetamiprid	0.43 ± 0.08aA	0.20 ± 0.06bA	0.52 ± 0.07aA
Indoxacarb	0.80 ± 0.19aA	0.10 ± 0.06bA	0.58 ± 0.11aA
Azinphos-methyl	0.65 ± 0.20aA	0.10 ± 0.05bA	0.60 ± 0.11aA

*Spinosad bait had premixed sugar.
Means followed by the same lowercase letter within a row or the same uppercase letter within a column are not significantly different (LSD test on transformed data, P > 0.05).

Table 2 Mean durations of feeding events ± SE (s) of *Rhagoletis indifferens* exposed to insecticides with and without 20% sugar over 5-min periods

Treatment	Nitrogen-starved flies: Treatment with sugar	Nitrogen-starved flies: Treatment without sugar	Fully-fed flies: Treatment with sugar
Water	37.8 ± 6.8aA	17.7 ± 8.4aA	48.3 ± 8.6aA
Spinosad bait*	39.0 ± 10.4aA	Not tested	39.0 ± 5.7aAB
Spinosad	33.2 ± 5.5aAB	16.7 ± 5.4aA	34.1 ± 11.4aB
Imidacloprid	17.6 ± 3.3aBC	3.8 ± 0.8bBC	41.8 ± 10.9aAB
Thiamethoxam	17.4 ± 3.4bC	10.5 ± 1.5bAB	28.8 ± 2.5aAB
Acetamiprid	7.5 ± 1.1aD	4.8 ± 1.7bC	11.1 ± 1.4aC
Indoxacarb	40.3 ± 9.0aA	21.3 ± 8.6aA	37.9 ± 8.7aAB
Azinphos-methyl	38.9 ± 5.2aA	23.0 ± 10.8aA	32.8 ± 5.2aAB

*Spinosad bait had premixed sugar.
Means followed by the same lowercase letter within a row or the same uppercase letter within a column are not significantly different (LSD test on transformed data, P > 0.05).

For feeding durations (table 2), there was an insecticide effect (F = 10.3; d.f. = 7, 315; P < 0.0001), a sugar in insecticides effect (F = 14.9; d.f. = 1, 315; P = 0.0001), no insecticide × sugar interaction (F = 0.8; d.f. = 6, 315; P = 0.5638) and a diet effect (F = 4.5; d.f. = 1, 315; P = 0.0338) with a near significant insecticide × diet interaction (F = 1.8; d.f. = 7, 315; P = 0.0784).

Feeding durations by nitrogen-starved flies on sugar water, spinosad bait, and spinosad, indoxacarb and azinphos-methyl with sugar did not differ, but were longer than on acetamiprid. Feeding durations by nitrogen-starved flies on insecticides without sugar and by fully-fed flies on insecticides with sugar followed a similar pattern (table 2). The presence of sugar increased feeding durations by nitrogen-starved and fully-fed flies only in the neonicotinoids and not in the other insecticides (table 2). Overall, fully-fed flies fed only for a slightly longer duration (34.2 s) than nitrogen-starved flies (29.0 s), but on thiamethoxam with sugar, the duration was 2.4 times longer in fully-fed than nitrogen-starved flies (table 2).

Mortality of flies exposed to insecticides

In experiment 2, there was no difference in paralysis/mortalities between sexes, so data from sexes were pooled (three-way ANOVA, e.g. at 24 h, nitrogen-starved flies, sex effect: F = 1.6; d.f. = 1, 106; P = 0.2030; fully-fed flies, sex effect: F = 0.1;

d.f. = 1, 107; P = 0.7637). Four-way ANOVA indicated that were significant effects of insecticide, sugar in insecticides, diet, and time (F = 41.8; d.f. = 6, 112; P < 0.0001, F = 68.2; d.f. = 1, 112; P < 0.0001, F = 28.3; d.f. = 1, 112; P < 0.0001 and F = 212.0; d.f. = 2, 224; P < 0.0001, respectively), but that there also were interactions among all factors (P < 0.05) except sugar × diet and insecticide × sugar × diet (P > 0.05).

Analyses of each factor separately (insecticide, sugar in insecticides, diet, and time) from the four-way ANOVA indicated significant effects of all factors. Insecticide effects ($t = -8.8$; d.f. = 12; P < 0.0001) were evident in that, overall, spinosad, thiamethoxam and azinphos-methyl caused the highest fly paralysis/mortalities (figs 1 and 2) (specific comparisons in next paragraph). There was an effect of sugar in insecticides on paralysis/mortality ($t = -8.3$;

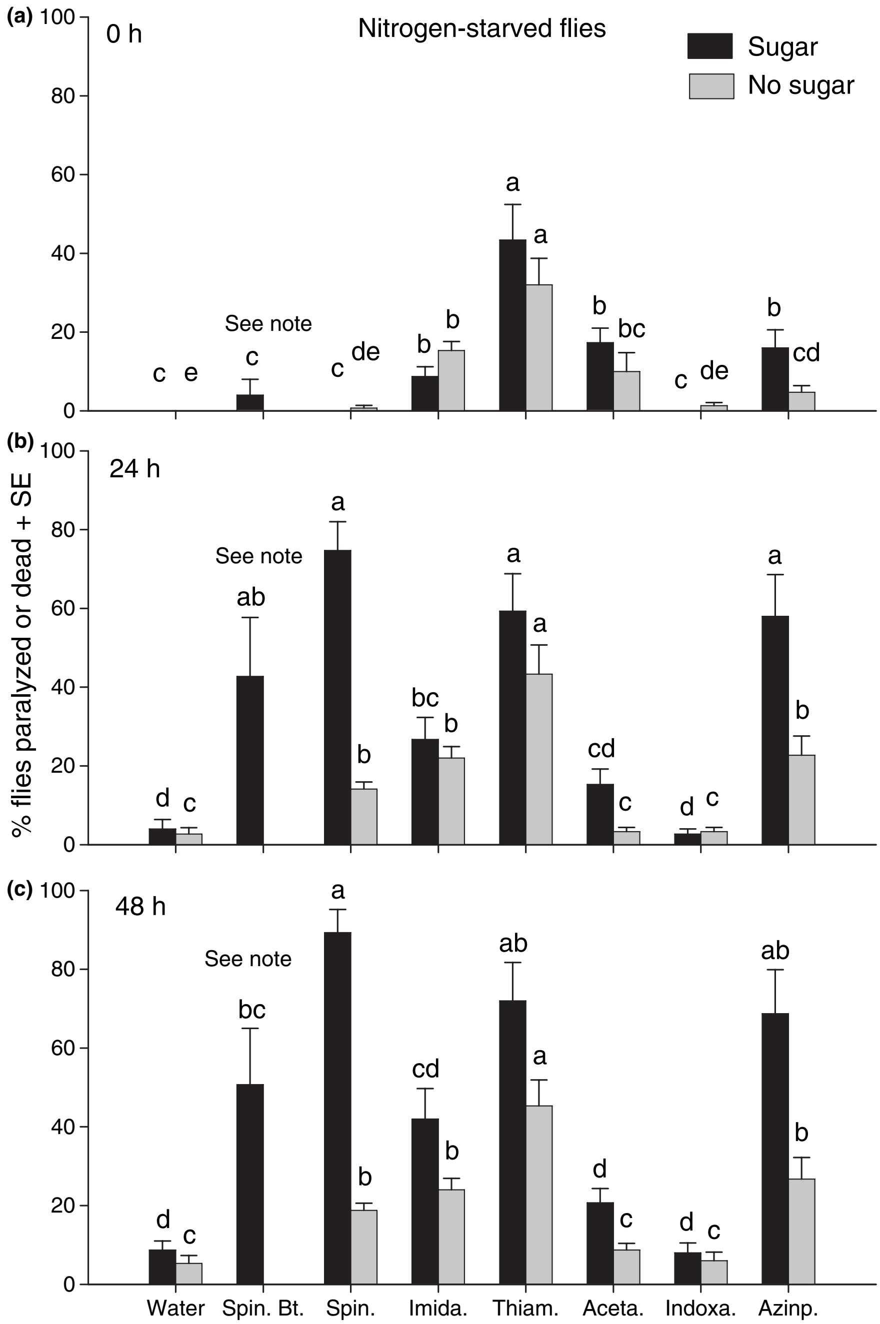

Fig. 1 Mean percentage of nitrogen-starved *Rhagoletis indifferens* paralysed or dead at (a) 0 h, (b) 24 h and (c) 48 h after exposure to insecticides with and with no sugar. Note: there was no spinosad bait treatment with no sugar. Means within sugar or no sugar groups with same letters are not significantly different (P > 0.05).

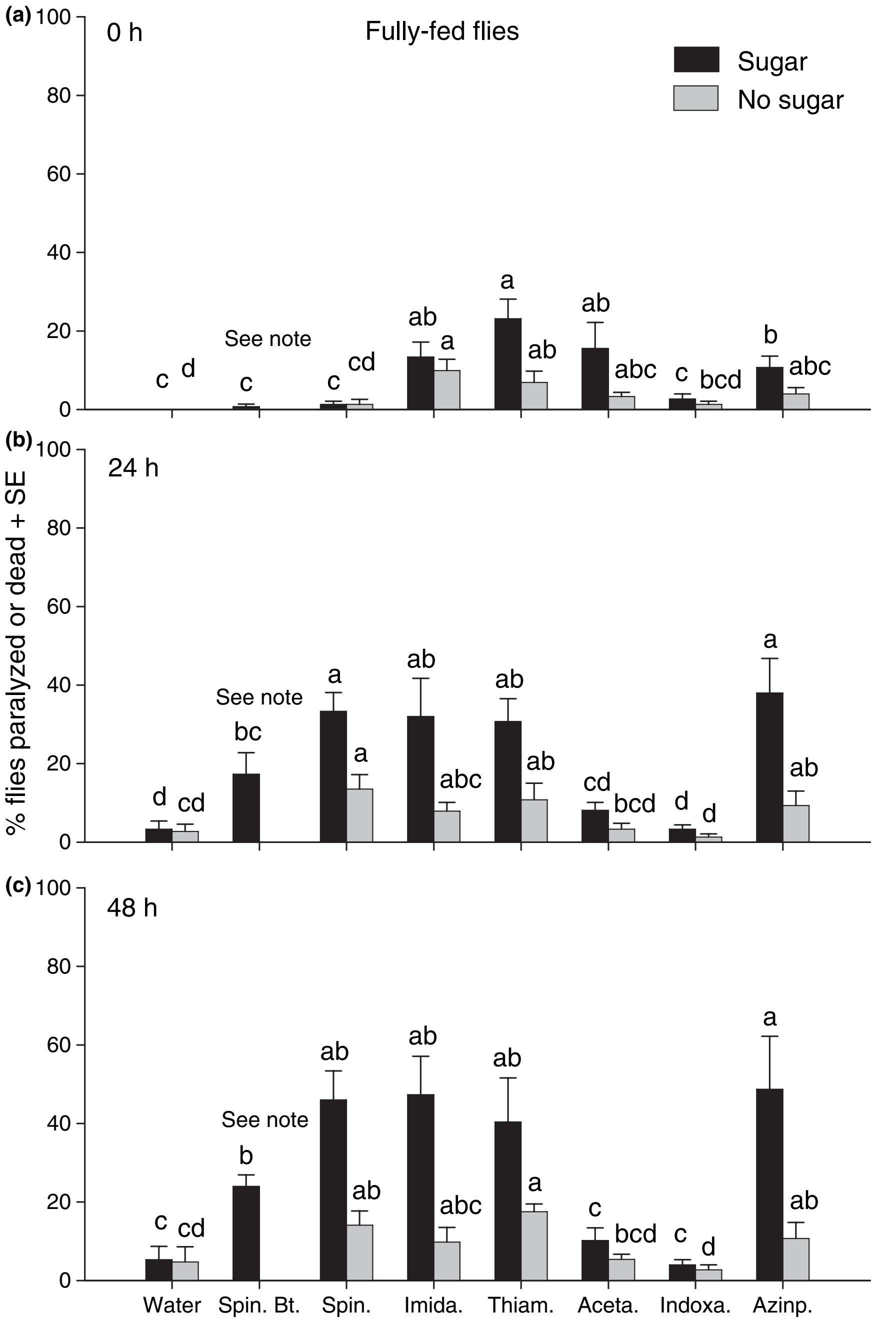

Fig. 2 Mean percentage of fully-fed *Rhagoletis indifferens* paralysed or dead at (a) 0 h, (b) 24 h and (c) 48 h after exposure to insecticides with and with no sugar. Note: there was no spinosad bait treatment with no sugar. Means within sugar or no sugar groups with same letters are not significantly different (P > 0.05).

d.f. = 112; P < 0.0001), as it was higher in flies exposed to all treatments with sugar (P = 0.0103–<0.0001) other than water and indoxacarb (P = 0.6867 and 0.7377, respectively) (figs 1 and 2). There was an effect of diet on paralysis/mortality (t = 5.3; d.f. = 112; P < 0.0001), as it was higher in nitrogen-starved than fully-fed flies in spinosad, thiamethoxam and azinphos-methyl treatments (P = 0.0107 to <0.0001), although not in water, imidacloprid, acetamiprid and indoxacarb treatments (P = 0.1564 to 0.7973) (figs 1 and 2). There were time effects (0 vs. 24 h, t = −17.1; d.f. = 224; P < 0.0001; 0 vs. 48 h, t = −19.8; d.f. = 224; P < 0.0001; 24 vs. 48 h, t = −8.7; d.f. = 224; P < 0.0001), as mortalities increased from 0 to 48 h (figs 1 and 2).

Paralysis/mortalities of nitrogen-starved flies exposed to different insecticides with sugar (including spinosad bait) at 0, 24 and 48 h (fig. 1) (F = 18.2; d.f. = 7, 32; P < 0.0001, F = 11.1; d.f. = 7, 32; P < 0.0001 and F = 10.8; d.f. = 7, 32; P < 0.0001, respectively) followed different patterns. Thiamethoxam had a greater effect at 0 h than all other treatments, but it had similar effects as spinosad and azinphos-methyl by 24 and 48 h. Spinosad bait had an effect that was in between this group and imidacloprid, acetamiprid and indoxacarb treatments at these times (fig. 1). Paralysis/mortalities of nitrogen-starved flies exposed to insecticides with no sugar at 0, 24 and 48 h ($F = 14.7$; d.f. = 6, 28; $P < 0.0001$, $F = 18.6$; d.f. = 6, 28; $P < 0.0001$ and $F = 14.3$; d.f. = 6, 28; $P < 0.0001$, respectively) followed similar patterns in that thiamethoxam had the greatest effect at all three times (fig. 1).

Paralysis/mortalities of fully-fed flies exposed to different insecticides with sugar (including spinosad bait) at 0, 24 and 48 h (fig. 2) ($F = 11.7$; d.f. = 7, 32; $P < 0.0001$, $F = 9.2$; d.f. = 7, 32; $P < 0.0001$ and $F = 7.3$; d.f. = 7, 32; $P < 0.0001$, respectively) followed different patterns. At 0 h, imidacloprid, thiamethoxam and acetamiprid were similar and more effective than spinosad bait, but by 24 or 48 h, spinosad bait did not differ from spinosad, imidacloprid and thiamethoxam (fig. 2). Paralysis/mortalities of fully-fed flies exposed to insecticides with no sugar at 0, 24 and 48 h ($F = 4.1$; d.f. = 6, 28; $P = 0.0046$, $F = 3.3$; d.f. = 6, 28; $P = 0.0144$ and $F = 3.8$; d.f. = 6, 28; $P = 0.0064$, respectively) were low and differed only slightly over time. At 0 h, imidacloprid, thiamethoxam and acetamiprid had greater effects than spinosad, but by 24 and 48 h, all four and azinphos-methyl did not differ (fig. 2).

Paralysis and recovery of flies after feeding on insecticides

In experiment 3 (table 3), mortality patterns of nitrogen-starved male and female flies caused by insecticides differed (insecticide: $F = 7.2$; d.f. = 5, 219; $P < 0.0001$, sex: $F = 14.3$; d.f. = 1, 219; $P = 0.0002$, insecticide × sex: $F = 2.2$; d.f. = 5, 219; $P = 0.0556$). Analyses within sexes separately (simple effect) showed that males fed longer on spinosad bait than on all treatments except imidacloprid. Females fed for similar durations on all treatments except acetamiprid. Females fed longer on spinosad and thiamethoxam than males ($t = 2.9$; d.f. = 219; $P = 0.0036$ and $t = 3.4$; d.f. = 219; $P = 0.0008$, respectively). Percent paralysis/mortality between sexes did not differ (Fisher's exact tests, $P = 0.3345$–1.000). There was no paralysis/mortality at 0 and 2 h of flies that fed on spinosad bait and spinosad. Imidacloprid, thiamethoxam and acetamiprid paralysed flies at 0 h, but at 2 h, flies recovered, with the highest recovery from acetamiprid (table 3). At 24 h, 100% of flies that had fed on spinosad bait were dead. Thiamethoxam caused the second highest mortality (table 3).

Table 3 Single feeding durations and mortality of nitrogen-starved *Rhagoletis indifferens* at three times after ingestion of insecticides with sugar

	Males		Females	
Treatment	N	Mean duration ± SE (s)	N	Mean duration ± SE (s)
Sugar water	18	15.7 ± 5.0b	21	16.5 ± 3.3a
Spinosad bait	18	20.8 ± 5.2a	21	21.3 ± 6.7a
Spinosad	19	7.3 ± 1.6bc	21	20.3 ± 4.1a
Imidacloprid	18	17.1 ± 7.9ab	22	19.0 ± 4.8a
Thiamethoxam	18	5.8 ± 1.3bc	22	18.5 ± 3.8a
Acetamiprid	17	3.6 ± 1.0c	21	5.4 ± 0.9b
	% flies paralysed or dead after:			
Treatment	N*	0 h	2 h	24 h
Sugar water	39	0c	0b	2.6e
Spinosad bait	39	0c	0b	100a
Spinosad	40	0c	0b	55.0bc
Imidacloprid	40	72.5ab	42.5a	37.5cd
Thiamethoxam	40	60.0b	56.4a†	72.5b
Acetamiprid	38	92.1a	23.7a	15.8de

Feeding durations within a column followed by same letters are not significantly different (LSD test on transformed data, $P > 0.05$). Percentage (%) flies paralysed or dead within a column followed by same letters are not significantly different (Tukey-type multiple comparison of proportions, $P > 0.05$). *Male and female data combined. †One missed observation ($n = 39$).

Discussion

Rhagoletis indifferens did not differ in percentages that fed on sugar water, spinosad bait, and the various insecticides with sugar, suggesting none of the materials was repellent. The lack of avoidance of insecticides is clearly important for their use in baits. However, in fully-fed flies, feeding duration was slightly higher on sugar water than spinosad with sugar, suggesting some flies preferred the taste of sugar water. In the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), addition of the organophosphate malathion did not affect attraction, but it deterred feeding (Prokopy et al. 1992).

Feeding durations of nitrogen-starved flies on imidacloprid, thiamethoxam and acetamiprid with sugar were shorter than on sugar water and spinosad bait, as the neonicotinoids paralysed flies quickly. Presence of imidacloprid in protein bait also resulted in less feeding than on control bait in *R. mendax* (Barry and Polavarapu 2005). Feeding duration of nitrogen-starved flies on acetamiprid with sugar was shorter than on imidacloprid and thiamethoxam with sugar, even though all neonicotinoids are insect nicotinic acetylcholine receptor agonists (Matsuda et al. 2001), indicating various neonicotinoids differ sufficiently in their specificity for receptors (Tomizawa and Casida 2005) to affect feeding.

Percent of flies that fed and numbers of feeding events were higher on insecticides with than without sugar. Sugar has no odour and likely did not affect how quickly flies found drops, but it clearly stimulated feeding after drops were found, after the sugar chemoreceptors on the labella or tarsi of flies (Frings and Frings 1955) contacted them. Flies responded equally to insecticides with 20% sugar and to spinosad bait, suggesting non-sugar components of the bait were not attractive and that the bait was not any more stimulating than sugar alone.

Nitrogen-starved and fully-fed flies exposed to most insecticides did not differ in immediate feeding responses. However, fully-fed flies fed longer on thiamethoxam with sugar than nitrogen-starved flies, reflecting perhaps an ability of fully-fed flies to withstand more feeding before effects of the toxin set in. Also, there was a diet effect on mortality after 2-h exposures to insecticides (below). Small energy decreases over 2 h may cause increases in hunger and thus increased feeding responses. It is also possible the 4–6°C higher temperature in test 3 than test 2 slightly increased activities of flies.

Paralysis/mortalities (= mortalities in discussion) in experiments 2 and 3 caused by spinosad bait and insecticides with sugar were time dependent, as spinosad bait did not paralyse flies as quickly as the neonicotinoids. In experiment 2, mortalities caused by spinosad bait did not differ from most other insecticides with sugar. This differed from experiment 3, where 100% of flies that fed one time on spinosad bait were killed, suggesting that in experiment 2 fewer flies fed on spinosad bait than on other insecticides. The relatively slow effect of spinosad bait may explain why flies can ingest it and still lay some eggs into fruit before dying (WLY, unpublished). The fast action of thiamethoxam may be one benefit of using it over spinosad bait or spinosad.

Mortalities in experiment 2 caused by spinosad, thiamethoxam and azinphos-methyl with or without sugar were also time dependent, but by 24 and 48 h they caused higher overall mortalities in nitrogen-starved flies than imidacloprid and acetamiprid. The results show that caution must be taken when interpreting immediate (0–2 h) effects of insecticides on fly mortality. Less toxic insecticides can paralyse flies more quickly than more toxic ones, but flies clearly can recover from paralysis caused by less toxic materials. Consistent with the present study and with knockdown effects of neonicotinoids on *R. indifferens*, spinosad was more toxic than acetamiprid and imidacloprid to *R. mendax* (Barry and Polavarapu 2005), and in *Rhagoletis pomonella*, spinosad (32 ppm) and thiamethoxam (100 ppm) caused similar mortalities and both caused higher levels than imidacloprid (11 ppm), at ~88, ~83 and ~55%, respectively (Reissig 2003). However, in contrast to the present findings, mortalities of *R. mendax* and *R. pomonella* exposed to thiamethoxam and imidacloprid mixed with sugar in latex paint on spheres were similar (Wright et al. 1999), but reduction in effectiveness after field ageing was greater in thiamethoxam- than imidacloprid-coated spheres (Wright et al. 1999; Ayyappath et al. 2000). The probable explanation was that more liquid was needed to mix thiamethoxam into the latex paint than imidacloprid, reducing the amount of latex that retained the thiamethoxam and resulting in rapid loss of its activity under heavy rainfall. If baits are applied weekly, however, chances of time-related activity losses would be reduced. Sublethal effects of insecticides also may need to be considered when evaluating their use in fly control because insecticides such as imidacloprid that have relatively low toxicity can reduce oviposition in *R. pomonella* and *R. indifferens* (Hu and Prokopy 1998; Yee 2008).

Mortalities of flies exposed to insecticides other than indoxacarb with sugar were consistently higher than to insecticides without sugar. This was likely caused by more flies feeding on drops with than without sugar. Mortalities of *R. pomonella* exposed to imidacloprid and thiamethoxam mixed with 20% sugar in latex paint on spheres were greater than to the insecticides alone (Wright et al. 1999). In *R. pomonella*, adding 1% and 5% sugar in spinosad on apples increased mortalities compared with spinosad alone, although adding 10% sugar surprisingly did not increase them (Reissig 2003).

The greater mortalities of nitrogen-starved than fully-fed flies were quite evident when flies were exposed to spinosad bait and to spinosad, thiamethoxam and azinphos-methyl with sugar. *Anastrepha ludens* (Loew) fed sugar only were attracted to nitrogenous volatiles (Robacker et al. 2000) and nitrogen-deprived *C. capitata* and melon fly, *Bactrocera cucurbitae* (Coquillett), were more likely to feed on nitrogen than nitrogen-fed flies (Vargas et al. 2002; Miller et al. 2004). This study suggests nitrogen-starved *R. indifferens* are also more likely to feed on sugar than fully-fed flies. Possibly nitrogen-starved flies were hungrier, sought nitrogenous sources, and were more active than nitrogen-fed flies, leading to more incidental contacts with insecticide drops. However, diet did not affect mortalities caused by imidacloprid, suggesting that a nitrogen-deficient diet increases mortalities only when the insecticides used are highly toxic.

Results have implications for managing *R. indifferens* using bait sprays. Thiamethoxam is comparable to spinosad in its effects on fly mortality, so using it with sugar may have similar results to using spinosad bait or spinosad. One benefit of using thiamethoxam with sugar may be that it kills flies more quickly, before they can oviposit, than spinosad bait. However, whether a fly feeds immediately after encountering a sugar-insecticide drop may depend on how much sugar or nitrogenous food it has eaten. If so, the abundance of food in trees needs to be considered when making decisions about treatment frequency or spray coverage.

Acknowledgements

I thank Katie Mackie, Robert Goughnour, and Janine Jewett (USDA-ARS) for invaluable assistance with tests, Dean Christie and Harvey Yoshida for supplying test materials, Roger Vargas (USDA-ARS, Hilo, Hawaii) and Carol Lauzon (California State University, East Bay) for reviewing earlier drafts of the manuscript, two anonymous reviewers for helpful comments, and the Washington Tree Fruit Research and Oregon Sweet Cherry Commissions for funding. This paper presents results of research only. Mention of a proprietary product does not represent an endorsement or recommendation for its use by USDA.

References

Ayyappath R, Polavarapu S, McGuire MR, 2000. Effectiveness of thiamethoxam-coated spheres against blueberry maggot flies (Diptera: Tephritidae). J. Econ. Entomol. 93, 1473–1479.

Barry JD, Polavarapu S, 2005. Feeding and survivorship of blueberry maggot flies (Diptera: Tephritidae) on protein baits incorporated with insecticides. Fla. Entomol. 88, 268–277.

Barry JD, Opp SB, Dragolovich J, Morse JG, 2007. Effect of adult diet on longevity of sterile Mediterranean fruit flies (Diptera: Tephritidae). Fla. Entomol. 90, 650–655.

DeLury NC, Thistlewood H, Routledge R, 2008. Phytotoxcity of GF-120® NF Naturalyte® Fruit Fly Bait carrier on sweet cherry (*Prunus avium* L.) foliage. Pest Manag. Sci. (URL http://www.Interscience.com) doi: 10.1002/ps.1644).

Duan JJ, Prokopy RJ, 1993. Toward developing pesticide-treated spheres for controlling apple maggot: carbohydrates and amino acids as feeding stimulants. J. Appl. Entomol. 115, 176–184.

Food Quality Protection Act, 1996. U.S. Congressional Record, vol. 142, 1489–1538.

Frings H, Frings M, 1955. The location of the contact chemoreceptors of the cherry fruit fly, *Rhagoletis cingulata* and the flesh fly, *Sarcophaga bullata*. Am. Midl. Nat. 53, 432–435.

Hu XP, Prokopy RJ, 1998. Lethal and sub-lethal effects of imidacloprid on apple maggot fly, *Rhagoletis pomonella* Walsh (Dipt., Tephritidae). J. Appl. Entomol. 122, 37–42.

Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB, 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacol. Sci. 22, 573–580.

Miller NW, Vargas RI, Prokopy RJ, Mackey BE, 2004. State-dependent attractiveness of protein bait and host fruit odor to *Bactrocera cucurbitae* (Diptera: Tephritidae) females. Ann. Entomol. Soc. Am. 97, 1063–1068.

Ministry of Agriculture and Lands, 2006. Tree fruit insect pests and diseases. Tree fruit leafrollers. http://www.agf.gov.bc.ca/cropprot/tfipm/treefruitipm.htm Accessed 28 May 2008.

Moreno DS, Mangan RL 2003. Bait matrix for novel toxicants for use in control of fruit flies (Diptera: Tephritidae). In: Invasive arthropods in agriculture. Ed. by Schwalbe C. Science Publishers, Inc., Enfield, NH, pp. 333–362.

Prokopy RJ, Papaj DR, Hendrichs J, Wong TTY, 1992. Behavioral responses of *Ceratitis capitata* flies to bait spray droplets and natural food. Entomol. Expt. et Applicata. 64, 247–257.

Reissig WH, 2003. Field and laboratory tests of new insecticides against the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). J. Econ. Entomol. 96, 1463–1472.

Robacker DC, Garcia JA, Bartelt RJ, 2000. Volatiles from duck feces attractive to Mexican fruit fly. J. Chem. Ecol. 26, 1849–1867.

SAS Institute, 2004. SAS/STAT® 9.1 user's guide. SAS Institute Inc. Cary, N.C.

Schabenberger O, 1998. Slicing interactions in SAS. http://www.stat.vt.edu/~oliver/SASSlice.html accessed 25 January 2005.

Thomas DB, Mangan RL, 2005. Nontarget impact of spinosad GF-120 bait sprays for control of the Mexican fruit fly (Diptera: Tephritidae) in Texas citrus. J. Econ. Entomol. 98, 1950–1956.

Tomizawa M, Casida JE, 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45, 247–268.

Vargas RI, Miller NW, Prokopy RJ, 2002. Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins, phloxine B and spinosad. Entomol. Expt. et Applicata. 102, 273–282.

Warner G, 2008. Bait is top cherry fruit fly spray. Good Fruit Grower Mag. 59, 28.

Washington State University, 2008. 2008 crop protection guide for tree fruits in Washington. EBO419. ECES Publishing and Printing, Pullman, WA. 96 pp.

Wright S, Chandler B, Prokopy R, 1999. Comparison of Provado and Actara as toxicants on pesticide-treated spheres. Fruit Notes 64, 11–13.

Yee WL, 2006. Feeding history effects on feeding responses of *Rhagoletis indifferens* (Dipt., Tephritidae) to GF-120 and Nulure. J. Appl. Entomol. 130, 538–550.

Yee WL, 2007. GF-120, Nu-Lure, and Mazoferm effects on feeding responses and infestations of western cherry fruit fly (Diptera: Tephritidae). J. Agric. Urban Entomol. 23, 125–140.

Yee WL, 2008. Effects of several newer insecticides and kaolin on oviposition and adult mortality in western cherry fruit fly (Diptera: Tephritidae). J. Entomol. Sci. 43, 177–190.

Yee WL, Alston DG, 2006. Effects of spinosad, spinosad bait, and chloronicotinyl insecticides on mortality and control of adult and larval western cherry fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 99, 1722–1732.

Yee WL, Chapman PS, 2005. Effects of GF-120 Fruit Fly Bait concentrations on attraction, feeding, mortality, and control of *Rhagoletis indifferens* (Diptera: Tephritidae). J. Econ. Entomol. 98, 1654–1663.

Zar JH, 1999. Biostatistical analysis. 4th edition. Prentice Hall, Upper Saddle River, NJ.

